TwinN - Build Stronger Crops

What is TwinN?

TwinN is a unique product that reduces the amount of N fertilizer needed and increases yields

- Freeze-dried microbial product – selected high performance *Diazotroph* species
  - Produced in modern, sterile fermentation facility
  - Every batch quality control tested by Australian Govt. labs (AIRG)
  - > 10^{11} cfu/ha - very high concentration of microbes
  - Reliable shelf life 12 mo – cool (4°C) storage
  - 1, 5, 10, 100 ha packs

Who uses TwinN?

- Conventional farmers wanting to reduce N use and increase profits
- Biological farmers wanting a reliable microbial N fixer and soil improver
- Organic farmers wanting improved N nutrition and better crop growth

Which crops?

- Blueberries
- Tree Fruits – apples, stone fruits
- Wine & Table Grapes
- Vegetables
- Intensive temperate and tropical pastures/forage/lucerne
- Broadacre/field crops – cereals, corn, cotton, rice
- Tropical tree fruits, avocado, bananas, citrus, coffee, tea
- Macadamia, pecan, almonds
- Sugarcane
- Others

How does TwinN work?

TwinN improves crop performance by three main mechanisms

1. Converts N_2 from the air into a **steady supply of plant available N** through the entire crop season
2. Produces **larger, more efficient root system** due to production of Plant Growth Factors (PGFs) gives improved capture of applied N fertilisers (= **improved N use efficiency**)
3. **Improved soil health and structure.** Longer term use of TwinN lowers soil disease pressure and **builds soil carbon. TwinN microbes assist in mobilising bound P**

How to apply TwinN?

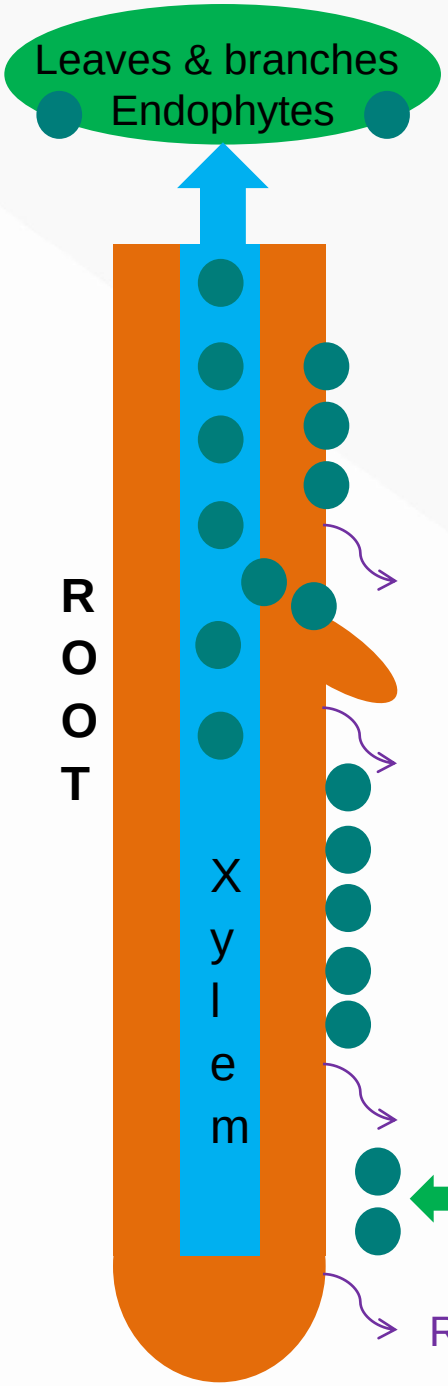
TwinN is applied to the roots where the microbes colonise, multiply and move all through the plant

Common application methods include:

- Drip, micro sprinklers or overhead irrigation systems – very convenient
- Trickle tape for vegetable crops
- Knifing into the root zone as for liquid fertilisers - common in cotton and sugarcane
- Spraying onto moist soil under trees – use a streaming nozzle and apply easily down the rows
- Delivery by flood irrigation for farmers who are set up to deliver urea by that method
- Boomspray onto wet soil or pasture

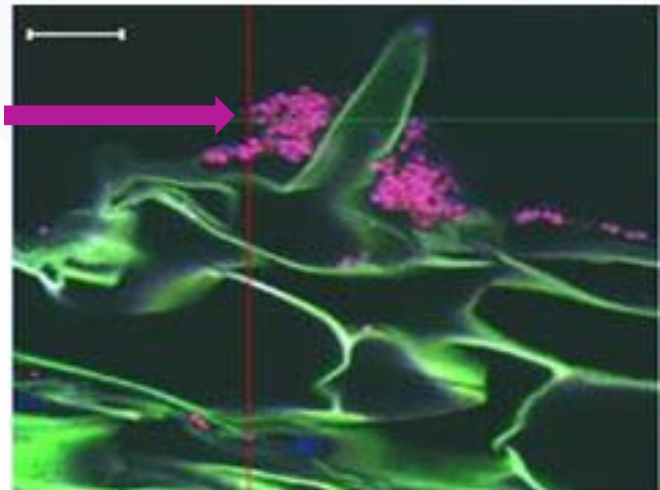
Application must deliver the microbes into a moist root zone for them to thrive

Where do the TwinN microbes act after application?



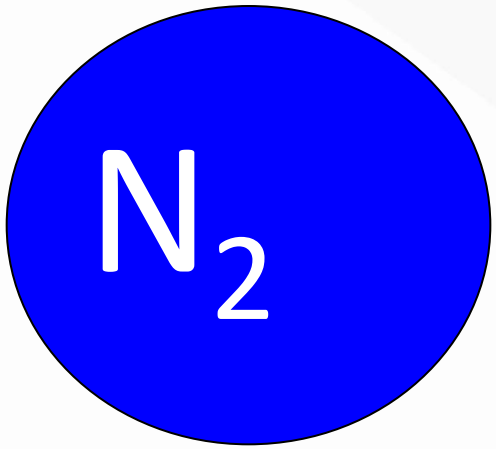
- When applied via soil application they colonise the rhizosphere – the zone of soil very close to roots. They also move up into the plant tissues and end up throughout the plant.
- TwinN microbes get carbohydrates and nutrients as root exudates and from plant tissues. In return they provide N, increase root efficiency and root/soil health – a true symbiosis

Azospirillum sp on rice roots



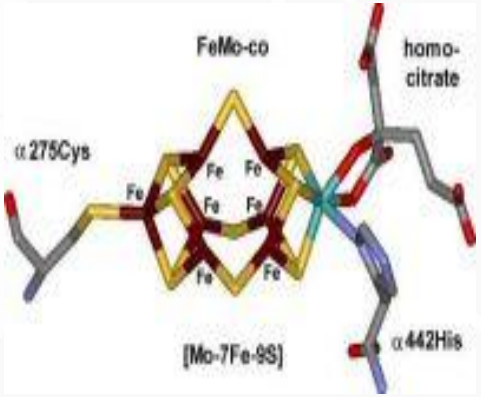
1st Mechanism of Action – N fixation

- **NH_3** is supplied steadily right through the season
- This helps keep Total Leaf N at optimum levels without high nitrates which reduce fruit quality and shelflife



From the atmosphere

N Fixing Bacteria



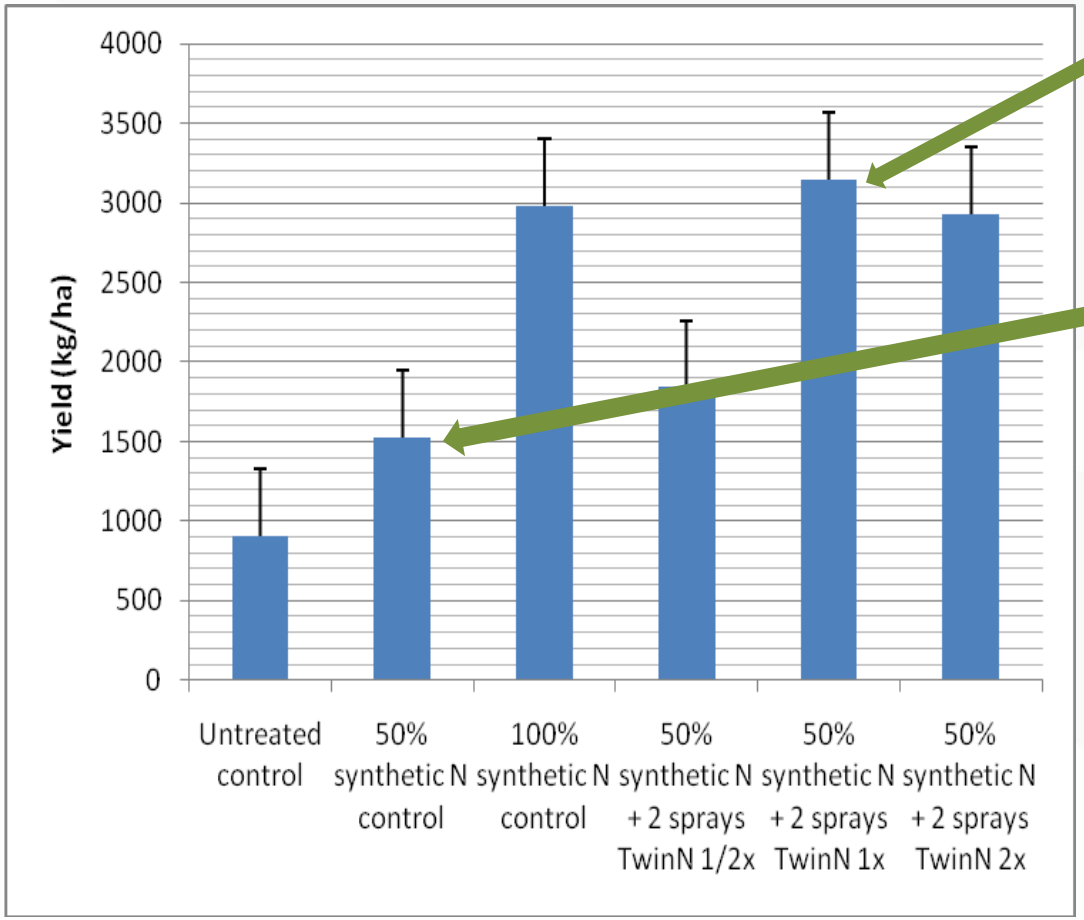
Nitrogenase enzyme

$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2$

= Nitrogen for crop

1st Mechanism of Action – N fixation

Wheat – South Africa – 2010 – Independent Registration Trial



- Full rate TwinN with 50% N gave the highest yield and was statistically equal to 100% N.
- 50% N with no TwinN delivered significantly lower yield (50% of control).
- 2X rate TwinN performed no better than 1X
- Half rate TwinN did not perform and is not recommended at all.

For most cereals we recommend a single TwinN and a 25 - 35% reduction in synthetic N, depending on N prices and value of the crop yield each season. TwinN gives farmers flexibility to adjust input costs and yield targets for each season.

2nd Mechanism of Action – larger, more effective roots

TwinN produces **larger root systems** due to auxin synthesis (IAA etc)

Note this picture is from a DPI-measured comparison and root mass rating increased >13% in TwinN plots.

This effect is mainly from increased root hairs increasing the root ball

- Greatly **increased root hair density** gives better nutrient capture of all nutrients
- **Improved capture** of mineralized and applied N **increases nitrogen use efficiency**
- More vigorous root growth **helps crops fight back from root damage** from pests and diseases

Improved N nutrition is due to a combination of N fixation plus better N use efficiency via the effect of TwinN on roots

3rd Mechanism of Action – Improved Soil Health

TwinN can reduce populations of some pathogenic microbes

- TwinN encourages growth of beneficial microbes that help keep soil pathogens in check
- See trial data on www.mabiotec.com for effects of reduced *Phytophthora* and *Fusarium*

TwinN increases root nodulation in legumes

TwinN enables reduced N application rates

- Lowers impact of urea on soil organic carbon
- Avoids problems with soil pH
- Lowers impact of synthetic N on soil structure

TwinN increases nutrient availability

TwinN microbes release organic acids **improving availability of P (P solubilisation)** and increase the **availability of some micronutrients** in soils

Picture 9: Clover nodulation on 12 September 2009 (four months after application of TwinN and nine months after trace elements)

Picture 8: Original clover nodulation, 11 December 2009

© 2009 Vital Soil Consulting

Why use TwinN?

1. Improve profitability
    a) Decrease input costs from nitrogen fertiliser (when N prices are high and crop prices are low)
    b) Increase yields (when crop values are high)

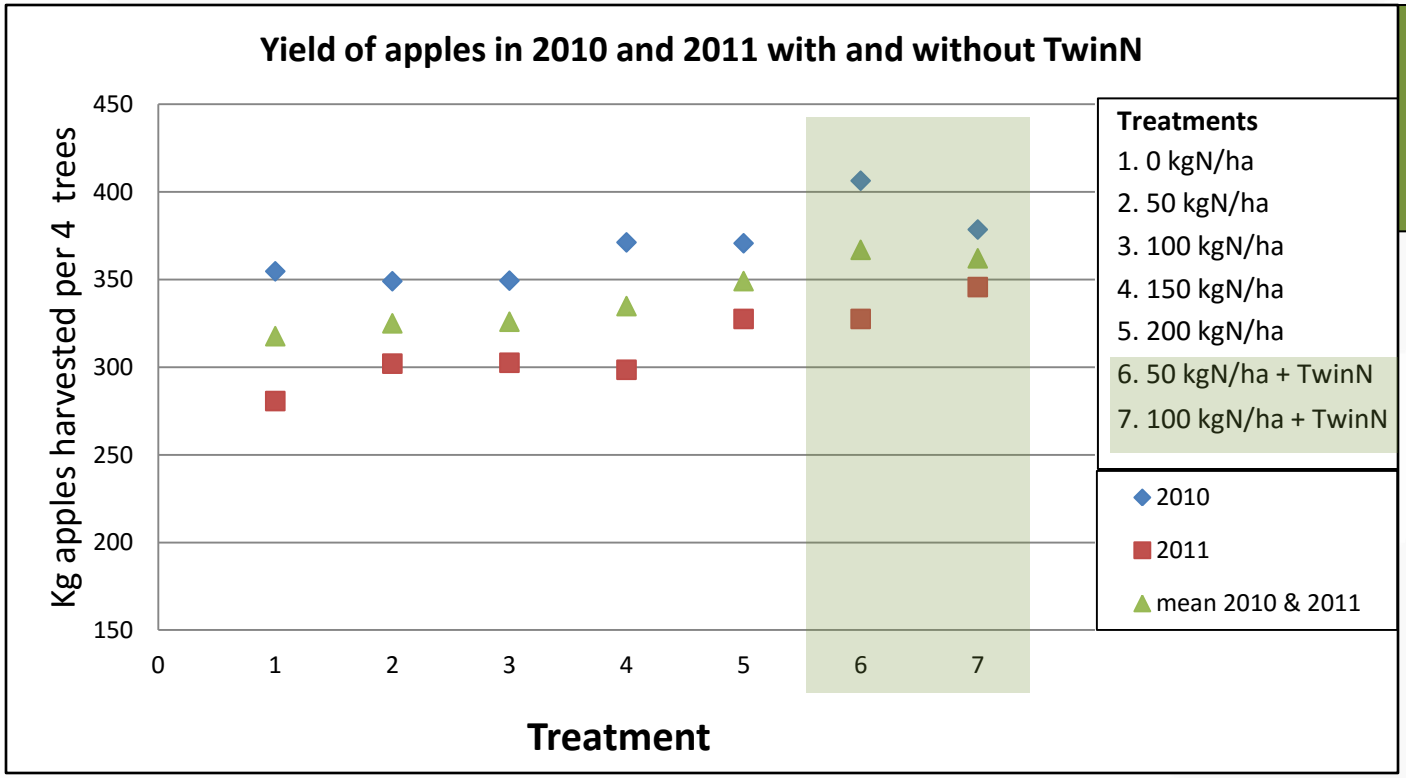
Farmers can select between a) and b) according to crop prices and fertiliser prices. For high value/ high yield crops apply TwinN on top of the standard program or use a small N reduction.

2. Increase sustainability of production
    a) Improve soil productivity and crop vigour
    b) Decrease synthetic nitrogen effects on the environment
    c) Decrease carbon footprint of production (eg bio-ethanol)
3. Enable compliance with various legislations to limit nitrogen fertilizer applications – without the normal yield penalties from reduced N applications

Apple, Nectarine & Peach

- TwinN is used to enable 20 – 40% reduction in N up to a maximum cut of 50 U N/ha
- Spread cuts across the season
- Apply in spring and again before trees cease growth at the end of the season if economics allow
- If fruit prices are low then make larger cuts in N to save input costs
- If fruit prices are high use smaller N cuts and apply TwinN x 2

Bulmer Cider, Independent replicated trial, Apples, UK, 2010 & 2011



TwinN was applied to soil via two applications per year in spring and late summer

This trial was conducted to test TwinN's capacity to increase profits via improved yields and reduced fertilizer costs.

Improved sustainability of production was also noted.

Conclusions

- TwinN plus 50 or 100 kgN produced the highest yield in 2010 & 2011
- TwinN plus 50 kgN/ gave a 12.6% yield increase over 100 kgN with no TwinN
- This translates to reduced N costs, increased returns, decreased C footprint (see next slide) and improved long term soil health due to reduced N fertilizer applications

Peach, nectarine & apple farm use - Oregon USA 2011

		3/6/2011	7/11/2011
Block	lbs N Soil	% Leaf N	% Leaf N
14 A Gala Apple Grower Std.	27	2.31	2.00
14 A Gala Apple Twin N	28	2.66	2.23
14 B Nectarines Grower Std.	55	4.92	3.84
14 B Nectarines Twin N	55	4.50	4.04
Block 21 Peaches Grower Std.	63	4.81	3.96
Block 21 Peaches Twin N	53	4.98	3.87
Block 15 A Jazz Apple Grower Std.	49	3.43	2.50
Block 15 A Jazz Apple Twin N	64	3.24	2.61

TwinN in commercial farm use in Oregon State, USA, Two drip irrigation applications of TwinN – spring and autumn
Approx. 40% N reduction

Data provided by GS Long Pty Ltd

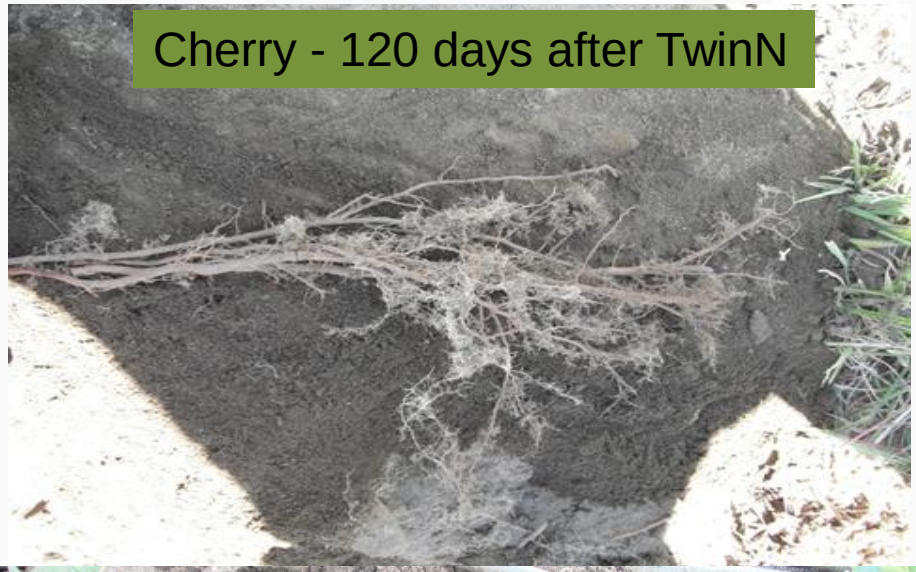
Peach, nectarine & apple farm use - Oregon USA 2012

		N	P	K	S	Ca	Mg	B	Mn	Zn
Apple Gala	Standard	2.1	0.17	1.74	0.13	2.78	0.6	25.9	105.4	15.9
	TwinN	2.38	0.2	1.57	0.15	2.39	0.54	24.2	83.9	15.8
Nectarines	Standard	3.88	0.35	2.6	0.37	1.84	0.59	28.5	45.4	47.3
	TwinN	3.09	0.22	2.07	0.19	2.24	0.66	27.3	46.9	26.1
Peaches	Standard	4.3	0.35	2.5	0.27	2.4	0.53	32.7	37.9	38.7
	TwinN	4.38	0.45	2.67	0.35	1.89	0.53	38.3	55.2	42.9
Apple Jazz	Standard	2.86	0.26	1.99	0.2	2.34	0.59	32.1	82.5	27.4
	TwinN	2.62	0.2	1.52	0.16	1.88	0.56	29.1	92.1	20.2

TwinN in commercial farm use in Oregon State, USA, Two drip irrigation applications of TwinN – spring and autumn
Approx. 40% N reduction

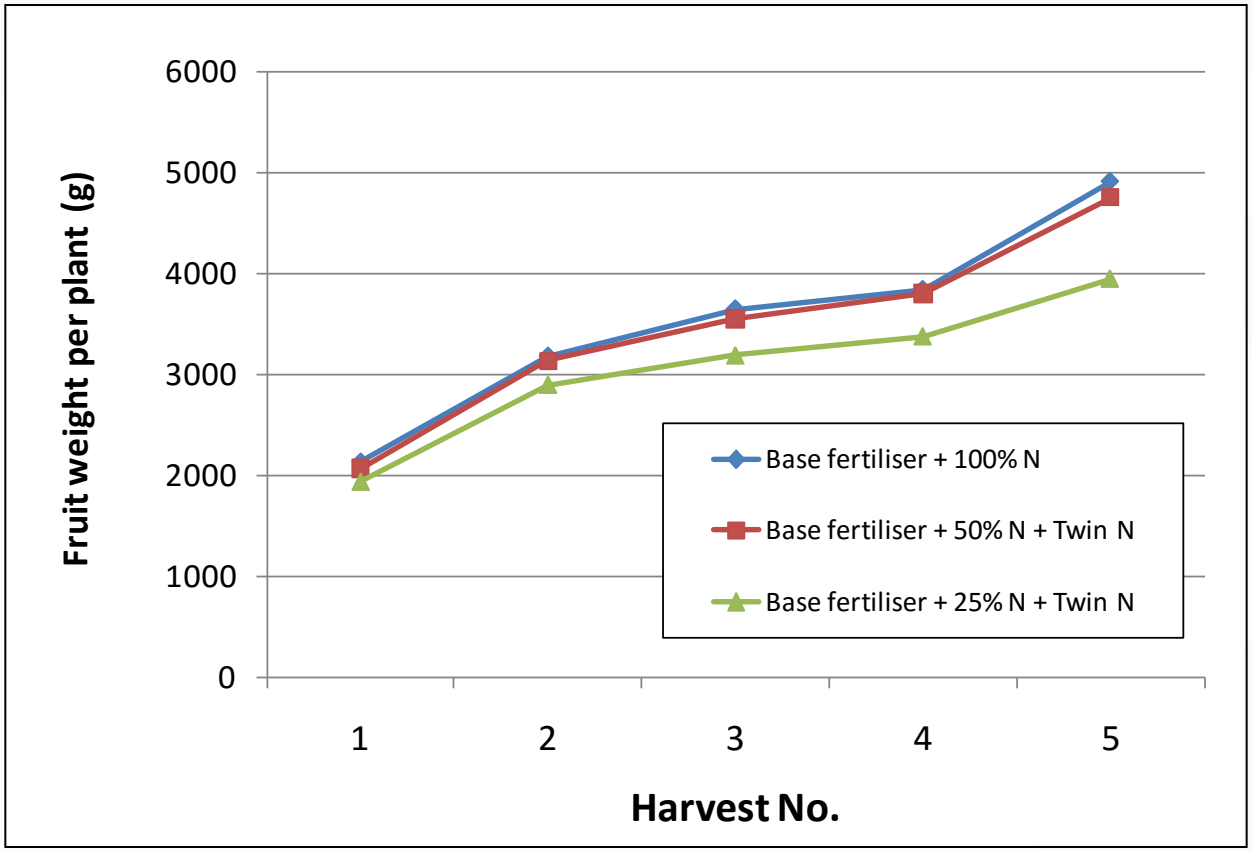
Data provided by GS Long Pty Ltd

Root growth effects – Chile 2010



Root growth between trees

Tomatoes – Bowen – QLD, Australia – 2009 - Trial by Peracto Ltd



TwinN was applied twice by trickle irrigation

- TwinN plus 50% N gave the same yield as 100%N (120 kgN)
- MAB recommends starting with 75% N plus TwinN to target a yield increase
- Growers like to reduce N late in the crop to guarantee fruit quality is high (Reduced nitrates in fruit improves storage and fruit texture)

TwinN Trial In Blueberries: Region Zonal VIII, Chile, 2013

•Trial Performed By Driscoll's de Chile S.A. In Collaboration With AMecological

•The trial was performed at a commercial blueberry farm on a 2006 planting of the cultivar Duke that averaged 10-11 T / ha. The soil was Tumao deep soil with slight compaction at 40 cm.

•Trial To Test

1. effectiveness of TwinN in growth of new shoots for fruit production

2. extent that TwinN produced blueberries could be stored for longer periods without loss of quality

TwinN Trial In Blueberries: Region Zonal VIII, Chile, 2013

Key Results

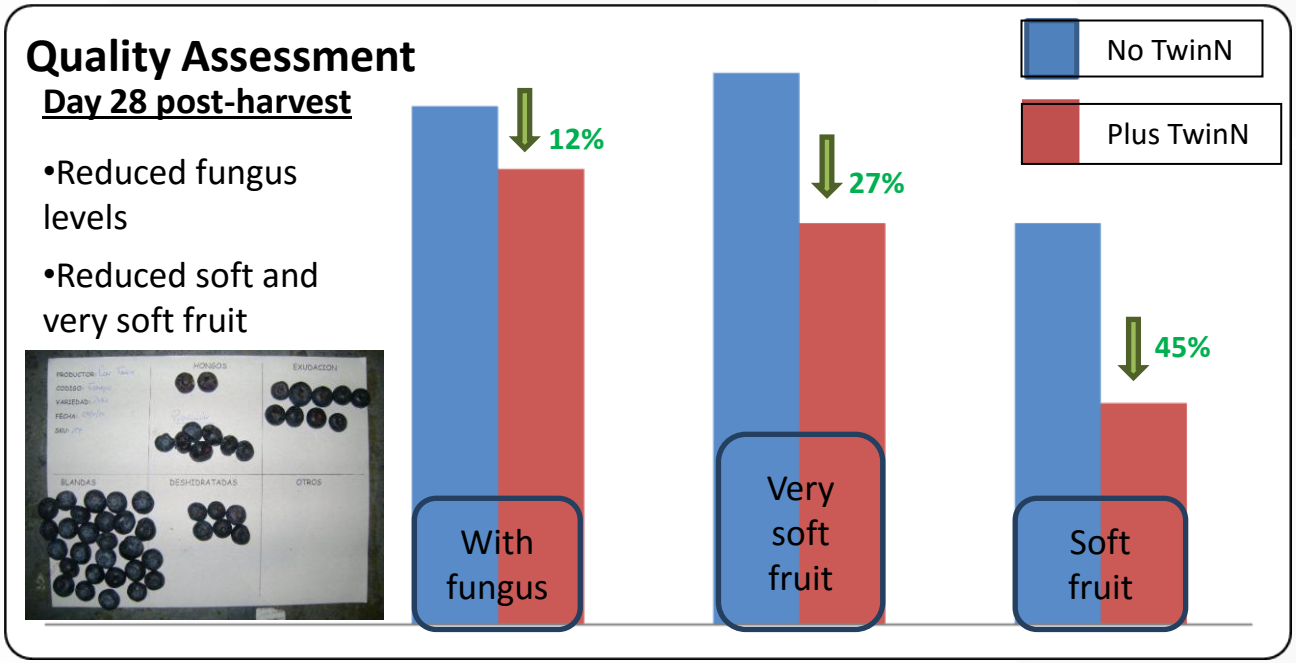
•TwinN treated plants produced a large increase in both the number and vigour of new shoots that farm managers expected to produce high yields in the next fruit season

•TwinN treated plants produced fruit that had significantly enhanced quality after four and six weeks storage compared to the conventional program

Crop Vigour Measurements		
	Number of shoots per year	Length of apical buds (cm)
With TwinN	126.4	54.2
No TwinN	62.4	36.2
Crop History: Planted 2006 (7 yrs old).		Average yield 10 – 11 tons/yr

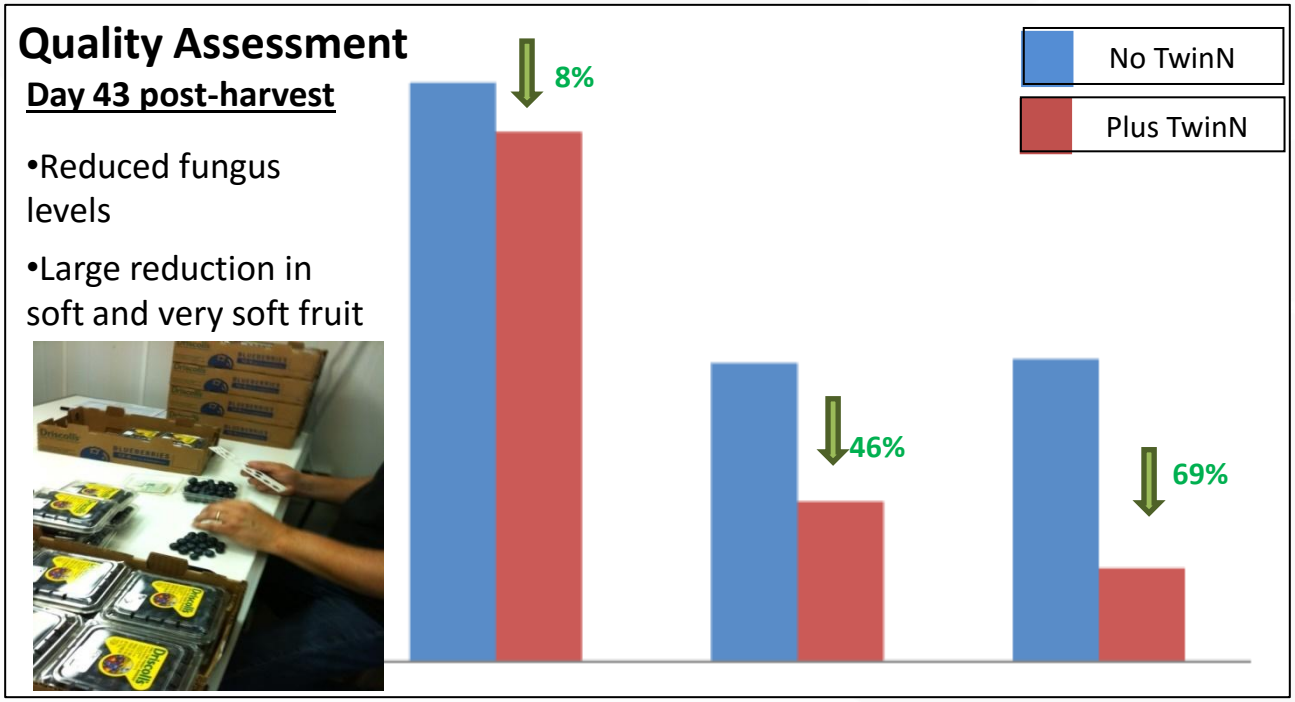
TwinN Trial In Blueberries: Region Zonal VIII, Chile, 2013

Fruit quality assessment at Day 28 after harvest showing TwinN substantially improved fruit quality after 28 days storage



TwinN Trial In Blueberries: Region Zonal VIII, Chile, 2013

Fruit quality assessment at Day 43 after harvest showing TwinN substantially improved fruit quality after 43 days storage



Result: -substantial quality improvement in TwinN treated plants
-rates of fungal spoilage reduced by 12% at 4 weeks and 8% at 6 weeks
-proportion of soft and very soft fruit reduced at 4 weeks and greatly reduced at 6 weeks after harvest

TwinN Demonstration In Blueberries: Abbotsford, BC, Canada, 2016

•Trial Performed By Fraser Berry Farms & PIER Management & Consulting Inc.

•The trial was performed at a commercial blueberry farm on a 1996 planting of the cultivar Duke. The soil is a silty loam with about 18% organic matter.

•**Objective:** - to test the capacity of TwinN to enable high yields and improve crops nutritional status

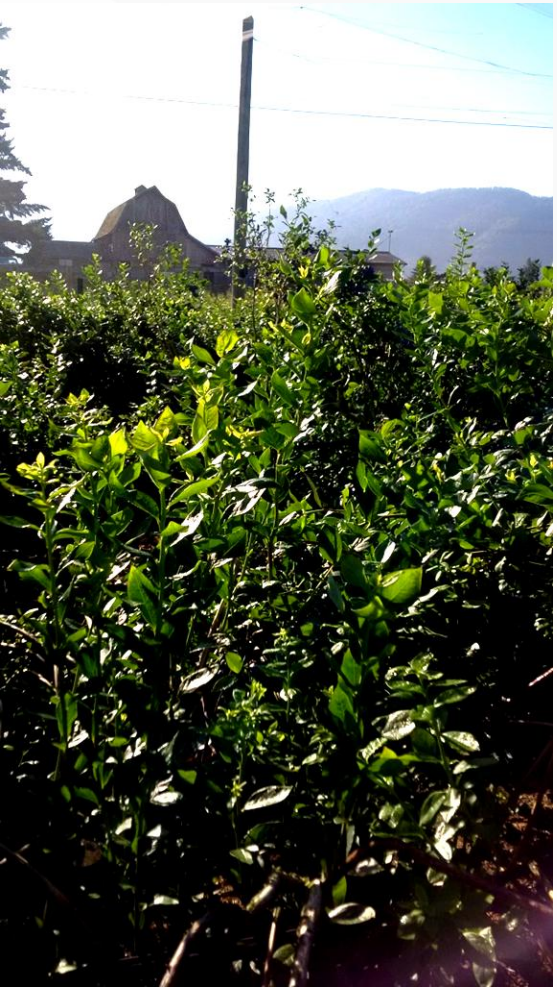
•**Additional Comment:** - the TwinN treated plot received a reduced total nitrogen application rate

TwinN Demonstration In Blueberries: Abbotsford, BC, Canada, 2016

Table 1: Summary of nutrient & TwinN applications and tissue test results.

April 13	Dry fertilizer applied to TwinN and Standard rows. N 158 lbs/ac (178 kg/ha), S 109 lbs/ac (123 kg/ha), Mg 28 lbs/ac (32kg/ha), K 172 lbs/ac (194 kg/ha), B 1.8 lbs/ac (2 kg/ha).										
May 5	Nitrogen14.13 lbs/ac (16 kg/ha) applied to TwinN and Standard rows.										
May 13	Nitrogen 20.22 lbs/ac (23kg/ha) applied to Standard rows only. Rows 1-10 received one application of TwinN										
May 21	Nitrogen 20.22 lbs/ac (23kg/ha) applied to Standard rows only										
	N	**P**	**S**	**K**	**Ca**	**Mg**	**Fe**	**Mn**	**Cu**	**Zn**	**B**
June 20 **TwinN**	2.34	0.17	0.17	0.51	0.45	0.18	36	43.4	2.8	24.3	30.3
June 20 **Standard**	2.35	0.16	0.10	0.51	0.44	0.15	37	52.4	2.3	16.3	36.6
Comment: Five weeks after TwinN application no major differences in tissue analysis											
July 31	Rows 1-10 received a second application of TwinN										
Aug 3 **TwinN**	1.68	0.12	0.15	0.49	0.75	0.24	66	88.2	3.0	18.6	71
Aug 3 **Standard**	1.60	0.11	0.14	0.38	0.68	0.21	55	81.9	2.9	19.2	68
Comment: TwinN plot shows N status 5% higher despite reduced N fertilizer, K status 29% higher, most other elements higher.											
Sept. 24 **TwinN**	1.83 +12%	0.12 +9%	0.10 +67%	0.62 +22%	0.7 +19%	0.20 +24%	43 +13%	55.2 +43%	72 +24%	51.1 +37%	145 +32%
Sept. 24 **Standard**	1.64	0.11	0.06	0.51	0.59	0.16	38	38.7	58	37.3	110
Comment: TwinN plot shows N status 11.6 % higher than Standard plot, all other elements significantly higher.											
Average **Twin N**	1.95	0.14	0.14	0.54	0.63	0.21	48	62.3	26	31.3	82
Average **Standard**	1.86	0.13	0.10	0.47	0.57	0.17	43	57.7	21	24.3	72

TwinN Demonstration In Blueberries: Abbotsford, BC, Canada, 2016



Summary Points

1. Row 1-11 (1 acre) received TwinN on May 13, 2016.
2. Untreated rows received 40.22 lbs / acre additional Nitrogen.
3. Visually, plants treated with TwinN had darker leaf color and appeared to have more new shoot growth from the base of the plants.
4. Nutrient testing shows similar nutrient levels between standard program and TwinN treated rows 3 weeks after application.
5. Five weeks after TwinN application no major nutrient differences shown in tissue analysis.

TwinN used in organic blueberries in Chile

On the left, no TwinN and nitrogen deficiency is evident.

On the right, 21 days after TwinN application and healthy leaf colour is regained

TwinN used in organic avocado, Australia

On the left, no TwinN

On the right, 2 months after a soil application of TwinN

Part of a trial by Avocado Australia

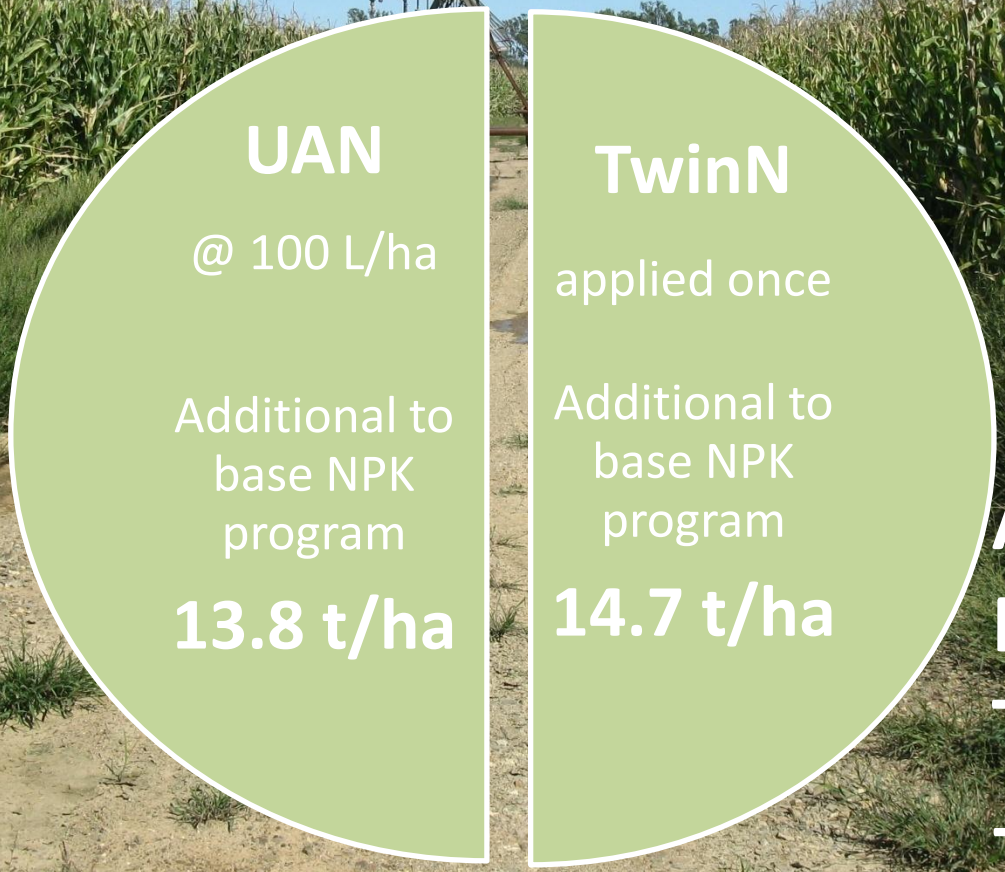
TwinN used in organic cherry in Chile

On the left, no TwinN

On the right, 120 days after TwinN application via fertigation. Roots showed significantly greater secondary root development

Farm Demonstration

- **Maize – Wagga Wagga - NSW- 2010**



Added Gross Profit from TwinN + $302/ha

Mapleton Agri Biotec

Using TwinN in potato

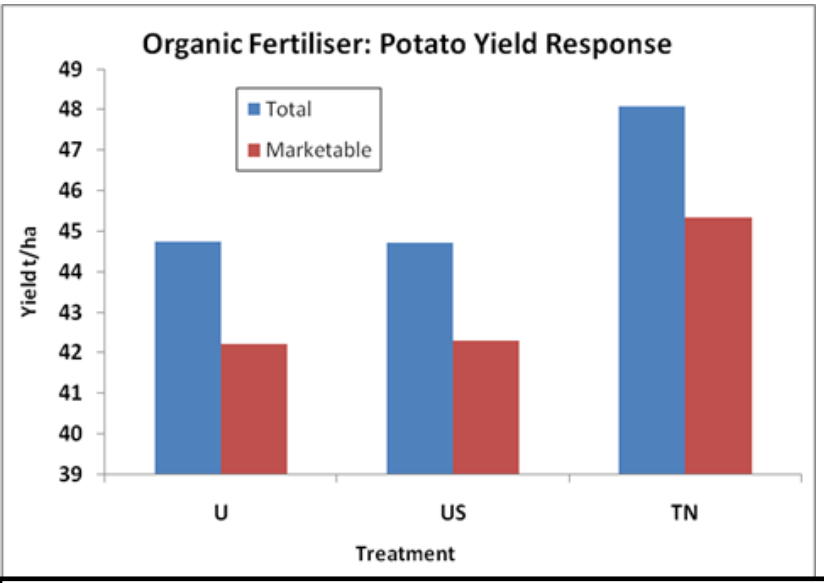
1. Keep plant / pre-plant N standard
2. Reduce total after-planting N by up to 50%
3. Reduce N evenly across the season (refer to the Tasmanian trial below for a good example of how to integrate TwinN into a nutritional program)
4. For organic crops apply TwinN plus the standard organic nutrients – do not reduce them
5. Apply TwinN once at 5 leaf stage (band onto rows)
6. Apply again 4 – 6 weeks later. For organic crops consider a 3rd application.
7. Apply by centre pivot (20 mm rain) or boomspray with very coarse or streaming nozzles onto moist soil. Microbes must be washed down into the roots to be effective.

Potato – replicated on-farm trial, Tasmania, by Serve Ag

- TwinN applied at 6 weeks post-planting, then at 9 and 15 weeks later
- TwinN plots received the standard preplant and at planting N programs
- TwinN plots received 125 kg/ha less of urea in season
- TwinN plots yielded 7.4% higher

Treatment	Urea	Urea/Kelp	TwinN/Reduced Urea	% increase over Urea
Total Yield T/ha	44.75	44.71	**48.08**	7.4%
Marketable Yield T/ha	42.21	42.29	**45.33**	**7.4%** **+ 3.12T/ha**

Potato – replicated on-farm trial, Tasmania, by Serve Ag

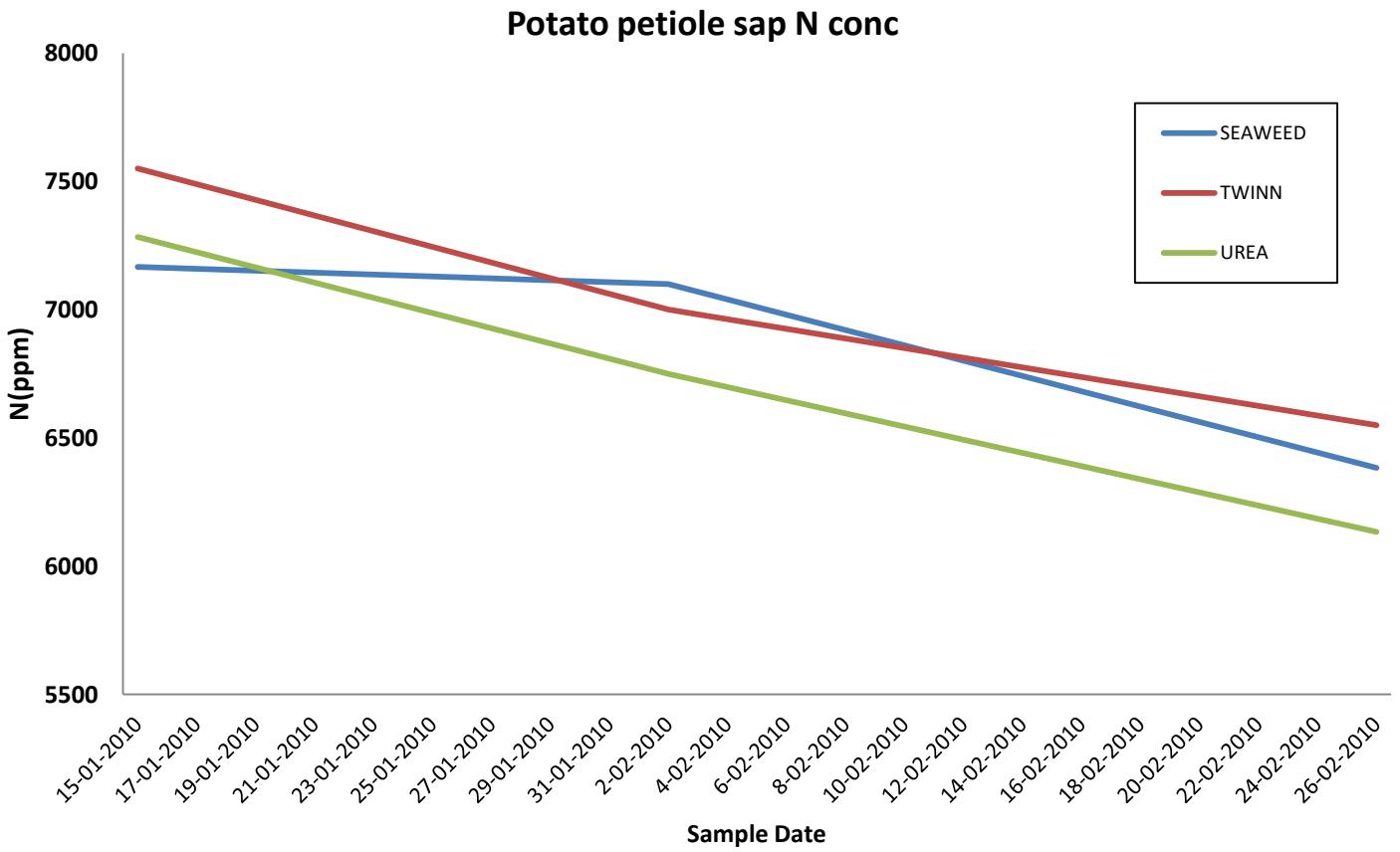


U = urea
US = Urea plus kelp
TN = 50% urea + TwinN

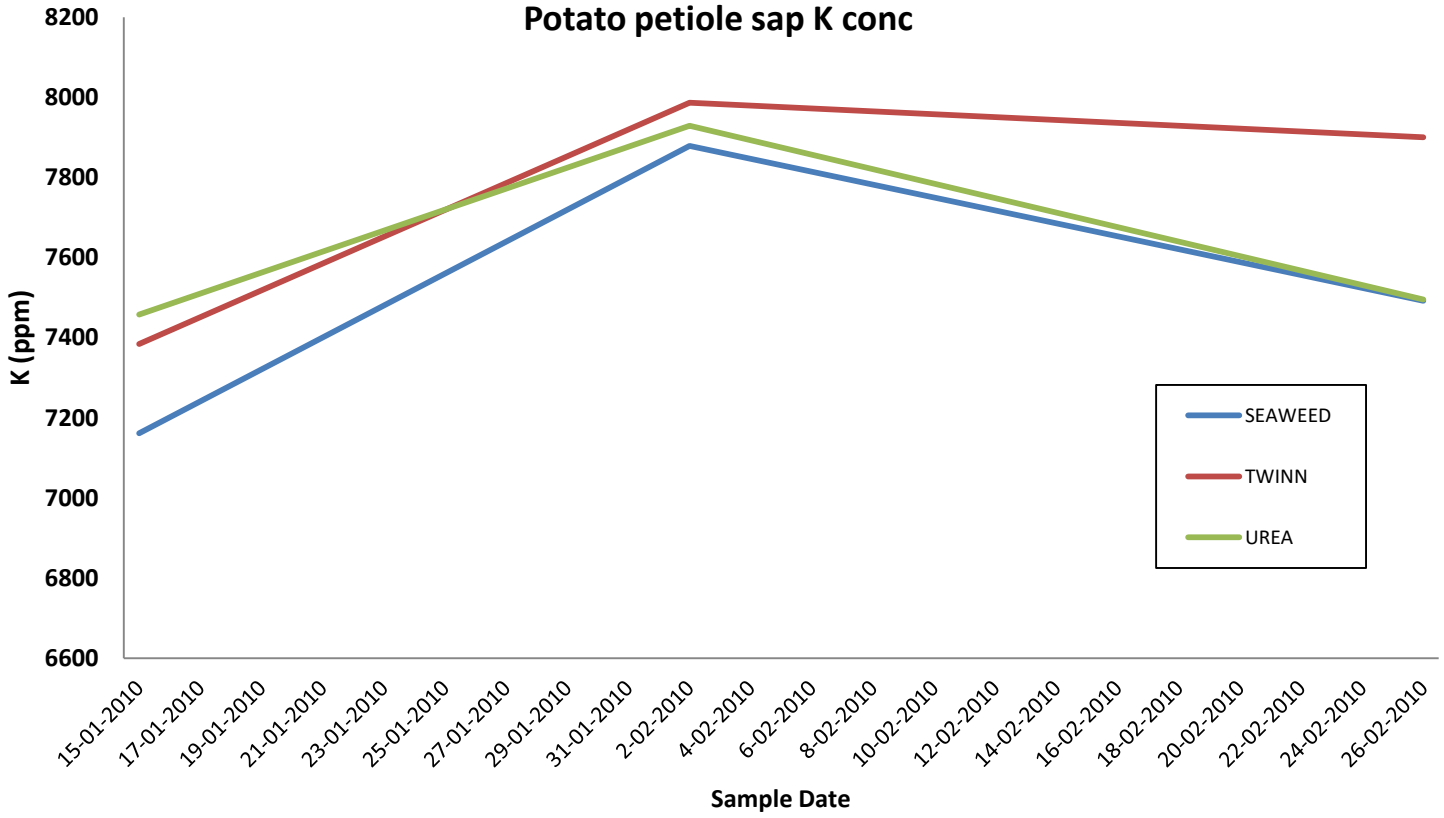
	Urea	Urea + kelp	Twin N
No. Stems	38.92	37.42	36.25
No. Tubers			
Mean no. tubers	33.03	32.58	31.11
Undersize	11.67	11.34	11.83
Marketable	67.42	72.42	69.92
Bonus	20	14.6	11.58
Weight of tubers			
Mean weight (kg)	5.35	5.11	5.16
Undersize (kg)	0.61	0.58	0.66
Marketable (kg)	10.13	10.15	10.88
Bonus	5.29	4.59	3.95

Potato Cultivar: Russet Burbank
Areas evaluated: 3 replicated plots 25m wide X 200m long
Soil Type: Ferrosol
Timing of application: Twin N applied at 6 weeks after planting, then at 9 and 15 weeks
TwinN application: Twin N in 160L water/ha by boom spray with coarse nozzles onto fully moist soil. Irrigated after application.
Pre Plant Fertiliser: 5-9-18-4 & trace at 1050kg/ha.
At Planting Fertiliser: 9-10-23-4 at 1000kg/ha
After Planting : All treatments received calcium nitrate at 100kg/ha at 4 weeks and at 50kg/ha at 10 weeks. All treatments received DAP, potassium sulphate and full trace elements at standard rate at 8 weeks. The control and kelp extract treatments received urea at 90kg/ha at 7 weeks and at 70kg/ha at 13 weeks.
The TwinN treatment received half the rate (50%) of each urea dressing.

Potato – replicated on-farm trial, Tasmania, by Serve Ag



Potato – replicated on-farm trial, Tasmania, by Serve Ag



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TwinN®

YIELD INCREASING MICROBES

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